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**Malcolm et al.**

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(54) **SINGLE-CHAIN RECOMBINANT  
COMPLEXES OF HEPATITIS C VIRUS NS3  
PROTEASE AND NS4A COFACTOR PEPTIDE**

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U.S.C. 154(b) by 0 days.

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Nov. 28, 1997.

(51) Int. Cl.<sup>7</sup> ..... **C07K 14/02**

(52) U.S. Cl. .... **530/350; 435/5; 435/69.7;**  
**435/219**

(58) Field of Search ..... **435/69.1, 320.1,**  
**435/252.3, 219, 69.7; 530/350; 536/23.2,**  
**23.72**

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(57) **ABSTRACT**

Covalent HCV NS4A-NS3 complexes comprising the cen-  
tral hydrophobic domain of native HCV NS4A peptide, a  
linker, and the HCV NS3 serine protease domain, wherein  
the hydrophobic domain of native HCV NS4A peptide is  
tethered by the linker to the amino terminus of the HCV NS3  
protease domain.

**11 Claims, 7 Drawing Sheets**

-continued

GGG CCA ACA CCC TTG CTG TAC AGG CTG GGA GCC GTC CAA AAT GAG GTC	1872
Gly Pro Thr Pro Leu Leu Tyr Arg Leu Gly Ala Val Gln Asn Glu Val	
610 615 620	
ACC CTC ACC CAC CCC ATA ACC AAA TAC ATC ATG GCA TGC ATG TCG GCC	1920
Thr Leu Thr His Pro Ile Thr Lys Tyr Ile Met Ala Cys Met Ser Ala	
625 630 635 640	
GAC CTG GAG GTC GTT ACG TAG	1941
Asp Leu Glu Val Val Thr	
645	

## (2) INFORMATION FOR SEQ ID NO:121:

## (i) SEQUENCE CHARACTERISTICS:

- (A) LENGTH: 36 base pairs
- (B) TYPE: nucleic acid
- (C) STRANDEDNESS: single
- (D) TOPOLOGY: linear

## (ii) MOLECULE TYPE: RNA (genomic)

## (xi) SEQUENCE DESCRIPTION: SEQ ID NO:121:

GCUCGCCCCG GGAUCCUCUA GGAUACACG UUCGAU	36
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## (2) INFORMATION FOR SEQ ID NO:122:

## (i) SEQUENCE CHARACTERISTICS:

- (A) LENGTH: 36 base pairs
- (B) TYPE: nucleic acid
- (C) STRANDEDNESS: single
- (D) TOPOLOGY: linear

## (ii) MOLECULE TYPE: RNA (genomic)

## (xi) SEQUENCE DESCRIPTION: SEQ ID NO:122:

CUAGAGGAUC CCCGGCGAG CCCUAUAGUG AGUCGU	36
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## (2) INFORMATION FOR SEQ ID NO:123:

## (i) SEQUENCE CHARACTERISTICS:

- (A) LENGTH: 21 base pairs
- (B) TYPE: nucleic acid
- (C) STRANDEDNESS: single
- (D) TOPOLOGY: linear

## (ii) MOLECULE TYPE: cDNA

## (xi) SEQUENCE DESCRIPTION: SEQ ID NO:123:

GCTCGCCCCG GGATCCTCTA G	21
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We claim:

1. A covalent HCV NS4A-NS3 complex comprising a central hydrophobic domain of native HCV NS4A peptide wherein the central hydrophobic domain comprises at least amino acid residues 22-30 of the native HCV NS4A peptide, a linker, and the HCV NS3 serine protease domain, wherein the hydrophobic domain of native HCV NS4A peptide is tethered by the linker to the amino terminus of the HCV NS3 protease domain.

2. The covalent HCV NS4A-NS3 complex of claim 1, wherein the linker comprises at least 4 amino acid residues.

3. The covalent HCV NS4A-NS3 complex of claim 2, wherein the linker consists of 4-6 amino acid residues.

4. The covalent HCV NS4A-NS3 complex of claim 3, wherein the linker consists of about 4 amino acid residues.

5. The covalent HCV NS4A-NS3 complex of claim 4, wherein the linker has a sequence defined by SEQ ID NO: 21 or SEQ ID NO: 22.

6. The covalent HCV NS4A-NS3 complex of claim 5, having an amino acid sequence selected from the group consisting of SEQ ID NOS: 1-20.

7. The covalent HCV NS4A-NS3 complex of claim 1 which is modified by replacement of one or more hydrophobic amino acid residues at position 17 or 18 of the HCV NS3 serine protease domain with a hydrophilic amino acid residue.

8. The covalent HCV NS4A-NS3 complex of claim 7 in which one or more isoleucine residues at position 17 or 18 of the HCV NS3 serine protease domain is replaced by a lysine residue.

9. The covalent HCV NS4A-NS3 complex of claim 8, having an amino acid sequence selected from the group consisting of SEQ ID NOS: 2-4, 6-8, 10, 12-14 and 16-18.

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**21** 10. The covalent HCV NS4A-NS3 complex of claim 1 which is modified by replacement of a serine residue at position 139 of the HCV NS3 serine protease domain with an alanine residue.

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**22** 11. The covalent HCV NS4A-NS3 complex of claim 10, having an amino acid sequence selected from the group consisting of SEQ ID NOs: 5-8, 15-18 and 20.

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